IV. *The effects of artificial temperature on the colouring of several species of Lepidoptera, with an account of some experiments on the effects of light.* By Frederic Merrifield, F.E.S.

[Read December 2nd, 1891.]

I have on previous occasions proved that both the spring and the summer emergence of *Selenia illustraria*, and the one emergence of *Ennomos autumnaria*, are materially influenced in their *colouring* by exposure of the pupa in its penultimate stage,—that is, in the one immediately preceding that in which the colouring of the perfect insect begins to show,—to a moderate difference of temperature, *i. e.*, within the limits of 57° and 80° F., the lower causing the greater darkness, and an exposure for a few days at the higher temperature being sufficient for its purpose. I have also proved that the *markings* of the former of these species, and probably those of the latter, may be very materially affected by long-continued exposure of the pupa in its earlier stages to a much lower temperature, one of about 33°.

Similar experiments have now been made on both emergences of the other two English species of *Selenia*, viz., *S. illunaria* and *S. lunaria*, with entirely similar results as regards colouring. These results are least marked in *lunaria*, as perhaps might have been expected from the fact that that species is in England generally single-brooded, and, where double-brooded, does not manifest as much dimorphism as its congeners do.

With respect to these *Selenias*, I now exhibit examples taken from as many as fifteen separate "families"—a term which I here use, not in its zoological sense, but as a convenient one for describing the offspring of a single pair. As the facts have now been established, and it would be exceedingly inconvenient and perhaps tiresome to show the very long series that these fifteen separate families have given me—more than 500 individuals—I have on the present occasion limited myself, in the case of the *Selenias*, to a selection of typical examples. But,

for the satisfaction of those who may not be conversant with all that has previously been established, it may be expedient to make the observations which follow, and which show the impossibility of ascribing the effects produced to any other cause than the temperature applied. I preface them with the remarks :—

(1). That the results lose much of their effect in consequence of being seen by artificial light. This reduces the effect in nearly all cases, and in some, where colour, and especially yellowish colouring, is concerned, makes a very great difference.

(2). That in all the *Selenias*, and in *autumnaria*, the under side is more affected than the upper; possibly this may have some significance in connection with the fact that these species, when at rest, expose only, or partly, their under sides.

(3). That in most of the species experimented on the male is more affected than the female; this, however, does not seem to be the case with *illustraria*.

(4). That the more vigorous and healthy the insect experimented on, the more strongly are the effects manifested on it.

Lunaria.—Two families, one of the spring, the other of the summer, emergence; 24 individuals. The markings of every one of those at the lower temperature are darker than those of any one at the higher temperature.

Illunaria.—Two families, one of the spring and one of the summer emergence, the former comprising 44 individuals, the latter rather more than 100. As to the former, I am not sure that all were from a single pair, but I think it nearly certain that they were so, for they came to me as a single lot, and where similarly treated show a close resemblance in appearance. These two families were exposed to several different temperatures—about 80°, 60°, 51°, and a somewhat lower temperature, *viz.*, that of the open air in winter and spring, emerging about April, when the temperature averaged little over 42°. Unless where the range of temperature was such as to cause little difference in colouring, which was the case as between some of the classes at the lower ranges, every individual which was kept at the lower temperature is darker than any which was kept at the higher temperature, with the exception of a few females, and these form no exception, if the under sides are looked to.

Moreover, when a considerable number of individuals, say, 10 to 20, were brought out at the moderately low temperature, the colouring as a whole, but not regularly, gradually darkens in proportion to the length of the exposure. I do not ascribe this directly to retardation, but to the fact that the physiological changes go on very slowly at the lower temperature; so that several weeks are necessary to produce as great an effect as several days at a forcing temperature would be sufficient for. A striking illustration of the delicacy with which the colouring responds when the temperature is applied precisely at the right time is afforded by the following circumstance. I had about a dozen of the summer emergence, which had been brought out at 80°, and, on looking them over after they were set, I noticed that three were decidedly deeper in colour than the rest. This perplexed me, until I remembered—and I found, on referring to my record, that I was right—that these three had been placed in the forcing-box at a separate time, and must have been more advanced when placed there, and consequently had been less exposed to the influence of the high temperature; for they occupied only from 3 to 5 days in emerging, instead of occupying 6 to 8 days, as the others did.

Of the spring emergence, it will be noticed that the first one was for as much as 14 days at a temperature of 80°, and it is much lighter than the rest of the family, which were not forced till February and March. It was one of six which were forced in November, the other five having died, an event which usually happens when the spring emergence is forced early in autumn.

Illustraria.—Five families, two of them (Families "T" and "R") of the spring emergence, comprising about 80 individuals. These were placed during their penultimate pupal stage at three different temperatures, *viz.*, at 80°, at about 60°, and out of doors (emerging April and early in May at a temperature averaging, during April, about 42°, or a little over). These last were generally darker than those at 60°. Every one of those at either of the lower temperatures is darker than any one of those at 80°, with the exception of one imperfectly developed specimen.

Three families ("I," "J," and "Red C") are of the summer emergence, and comprise more than 80 individuals. Each of these families was divided, and the

divisions respectively placed at two different temperatures, *viz.*, at 80°, and at about 46°. Every one of those (rejecting a few cripples), mostly among those at the higher temperature, at 46°, is darker than any of those at 80°.

A sixth family, of the spring emergence (Family "U"), numbering about 20 individuals, was exposed to several different temperatures for varying periods. These show the same general results, but of course not so definitely as the others ; moreover, they proved to be an unhealthy family, of which many died, or came out in a crippled condition. I exhibit typical specimens of each of these 6 families, also of 5 others, showing the same general results when exposed to similar conditions.

P. falcataria.—With an experiment on this I will conclude my summary of recent results, so far as concerns double-brooded *Geometræ*. I was supplied during the winter with a number of pupæ, which were very small and poor, as well as much tenanted by parasites, so that only 7 emerged. Three of these were placed, on the 19th April, at 80°, emerging in from 5 to 9 days, the others, left out of doors, appearing between the 21st and 29th May. With one doubtful exception those which emerged in the open air are manifestly darker and more strongly marked than any of those at 80°. I exhibit all.

V. urticæ.—In December, 1890, I exhibited a very dark specimen of this insect, the appearance of which I then thought, and have now no doubt, resulted from the exposure of its pupa to a temperature of about 47° for 5 weeks. I again exhibit this specimen, with a fair sample of 4 others of the same lot, all brought out at 80°, and emerging in about 6 days (Family "Y"). During the last summer I have experimented on three different families (or rather batches, each collected at the same time, and in similar localities). The first two, which I will call "V" and "L," were very kindly given to me by Mr. Vine ; "V" about the end of June, to the number of more than 100. I was under the impression, derived from my experiments with the *Geometræ*, that any change in colouring would be produced during one of the later pupal stages, and therefore took no pains to place the pupæ while quite fresh in the refrigerator ; and this may have been the cause of the comparatively slight effect produced on most of them, that effect becoming,

however, decided when the exposure had been of long continuance. Twelve were forced, of which eleven, of very uniform appearance, emerged in from 5 to 6 days. I exhibit four of these, which are not extreme, but a fair sample. From 42, which emerged after an exposure to about 47° of from 2 to 58 days, and which, though varying *inter se* in a moderate degree, are in general but slightly darker than the forced ones, I have selected the five darkest, each of which is darker than any of those forced. Nine others, which emerged after an exposure of from 60 to 67 days, present a very different appearance from the rest, mostly darker. I exhibit four typical specimens of these.

The next family, which I call "L," were given to me at the beginning of September. These were mostly exposed within a few hours after pupation, eight of them were forced, and of these I have selected four fair samples. Of the remainder, placed at 47° for about 20 to 42 days, 17 emerged, and the great majority of these present a peculiar appearance, mostly in the direction of darkness. I exhibit the 10 most peculiar out of the 17.

Another family, fine pupæ not more than a few days old, were given to me by Mr. Fletcher on the 22nd September; a very late brood. These I call the "M" family. Five were placed at 80°, emerging in from 6 to 7 days, and I exhibit them all; 13 emerged out of doors in from 3 to 5 weeks at an average temperature of about 57°, and I exhibit the seven most strongly coloured and marked of these. Others were exposed to a temperature of about 47° for from about 3 to 7 weeks, and I exhibit all of those which emerged, four in number; they are very different from the others, the general tendency being to greater darkness.

The general observations I have to make on the results of the *V. urticæ* experiments are as follows: The effect of exposing this species to cold for a moderate period seems to be generally, not universally, slightly to darken the colouring, to lower the colour of the yellow parts (in one individual this is almost gone), and to intensify the contrasts of light and shade; to spread the dark portions, and especially the dark outer margins, and, above all, the blue crescents contained in these. I have examined some long series of *V. urticæ* in other cabinets, selected to represent all ordinary variations, and have scarcely,

if at all, seen among them such conspicuous blue crescents as several of mine exhibit. The effect of exposure to cold continued for a period of from 8 to 9 weeks (or for a shorter period if exposed when the pupa is freshly formed) is more marked, generally in the increase of darkness. Further experiments must be tried on this insect, on which I am inclined to think the treatment most likely to produce darkness would be exposure for a considerable time from a very early pupal period to a temperature of about 50°.

I may add that I am well aware that my exhibits of *V. urticæ* would have been more effective had they been limited to a few extreme examples, but I have been desirous of imparting further knowledge than an exhibit so limited could have conveyed. Some of the most extreme specimens make a near approach to the var. *polaris* of Northern Europe.

Bombyx quercus and var. *callunæ*.—I have not been able to complete my experiments on these, but have obtained some results from temperature, and they are in the same direction as those obtained as to other species. To begin with the true *quercus* form, "*Family A*." Some recent pupæ and nearly full-fed larvæ, all stated to be from the same hedge at Windsor, supplied to me by Mr. Edmonds in 1890, were placed at 80°, generally at about a week after "spinning up," but some certainly at a somewhat later stage. I produce seven that emerged in from 29 to 40 days; they are fair samples of the seven, and are, as will be seen, very light coloured. "*Family B*." A second lot of 18 were sent me during last summer, also from Windsor. They were a particularly fine and healthy lot; some of them could not have been very recently "spun up," judging from the time when they began to emerge at the low temperature of about 47°, at which they were placed. Fifteen males emerged in from 39 to 71 days. The first was slightly crippled, owing to the want of proper provision for its reception. I produce the second at 40 days, and the fifteenth at 71 days. Both are, I think, rather dark, but the latest to emerge is substantially darker than the other, especially in its light parts. The 12 intermediate ones emerged in periods ranging from 40 to 70 days. These vary slightly, not darkening regularly, but there is on the whole a tendency, more particularly in the

light band, to grow darker as the length of the period of pupation, and consequently of exposure to the low temperature, increases.

Var. *callunæ*. "*Family A.*"—A few sent from Aberdeen were placed at 80°, and two emerged in from 27 to 46 days. I exhibit these, which are a good deal lighter than usual, especially the female, which can scarcely, if at all, be distinguished from the southern form. Another *family*, "*B*," from Perth, similarly forced, produced three males and two females. These are darker, but light for *callunæ*. I produce a fair sample of each sex; the males exhibited are perhaps slightly lighter than the average of the three. Another, *family* "*C*," from Perth, was divided; five males and five females were forced at 80°, appearing in from 29 to 42 days, and I exhibit two pairs of these—fair samples—one of the males slightly lighter than the average of the five. Six males and nine females were placed in the open air, and emerged in June and early in July; these varied but little. I produce two pairs which emerged between 28th June and 1st July, fair samples of the 11, except that one male is slightly darker than the average of the six males. It will be seen that the males especially are darker than those which had been forced.

To sum up as to *quercus* and *callunæ*. The same general result which has been noticed in other cases obtains here; that is, those at the higher temperature are lighter than those at the lower temperature. This particularly applies to the males, the females varying less; but in both males and females the forced ones have a reddish tint, which is wanting in the others. In some cases the effect of temperature is so considerable that I think some of the forced *callunæ* would, so far as regards colouring, be classed as *quercus*, while the individual *quercus* brought out in 71 days at the lower temperature is very dark for *quercus*.

I think these experiments tend to show that the southern form and its northern var. are respectively varieties of so fixed a kind when they reach the pupal stage that it is probably only in exceptional instances, if at all, that temperature could convert the one form, so far as its appearance is concerned, into the other. But further experiments should be made, especially in the application of a low temperature to the southern form

while freshly pupated. I believe temperature applied early in the larval stage has effected a complete conversion as regards habits, but I do not know whether it has had this effect on the colouring.

C. caja.—Dr. Chapman kindly sent me, at the beginning of February last, a batch of eggs which had been laid by a female in captivity. These I forced, and had from them more than 100 pupæ between the 12th and 24th March. Some of these were forced at 80°, some placed in the refrigerator at about 47°, a few more at 33°, and others at a temperature varying from about 50° to 60°. Those at 33° died after a time, those at 47° either died or emerged in a very crippled condition, many of those at 50° to 60° died, but of those at 80°, about 30, nearly all emerged, with no cripples. Unfortunately all but one of these last-named were males, while most of the others which emerged were females; so that the means of comparison are not so exact as might have been desired. Still, they show unquestionable differences corresponding with their treatment. In the fore wings of those forced the brown ground colour is paler, the colouring of the hind wings is a yellower orange, and their dark spots are smaller, and show a less tendency to coalesce. But the most striking feature is in the black bars across the abdominal segments; these, with scarcely an exception, are much longer and broader in the moths from the pupæ kept at the lower temperature than in those from the higher temperature. I exhibit three average samples of the forced males and the one forced female, and two males and two females of those at the lower temperature—average examples.

These experiments having indicated that *caja* flourishes under a high temperature, I placed some more pupæ, afterwards kindly sent me by Dr. Chapman, at the temperature of 80°—90°; the effect was to increase sensibly the pallor of the brown part of the fore wings, especially towards the outer margin, where the colouring assumes a clouded and blotchy appearance. I had five of these, and exhibit two of them, typical specimens, or only slightly paler than the average of the five.

Size and shape as affected by temperature in the pupal stage.—This is a subject I approach with some hesitation, because accidental circumstances have interfered with the completion of the crucial tests I meant to have

applied, and which are now in progress; but the evidence is so strong that I do not like to delay calling attention to the point, with a view of, if possible, enlisting other observers, until the question shall have been decided by exact measurements. I think it is generally assumed that, once the pupal state is entered upon, the size and shape of the imago are fixed once for all (except where the wings afterwards expand imperfectly), and no doubt that is true to the extent that, in an insect of variable size, the size the larva attains is the chief factor in the size of the imago, and under ordinary circumstances the only one. But there is very strong evidence that the size may be affected by the circumstances surrounding the pupa. The impression that this is so can hardly fail to be gathered by any one who sees the long series I possess of insects differently treated in the pupal stage, and I think I may say that this is the impression actually produced on all my friends who have seen these long series. It is an effect which by no means appears to operate in the same direction in all species.

In the three *Selenias*, of both the spring and the summer emergences, those at 80° appear smaller than those kept at and under 60°; it seems to be the same in *falcataria*, as well as in *B. quercus* and its var. *callunæ*. In *V. urticæ* there appears little difference, if any, in the size of butterflies from pupæ at 80° and from pupæ at about 60°; but those from the pupæ kept at 47° are generally smaller. I may add that on consulting the record of experiments on *P. pharos*, as recorded in 'Weissmann's Studies in the Theory of Descent,' by Meldola, I find it stated that the butterflies from three pupæ which had been iced were sensibly smaller than the rest.

As to *shape*, I can only say at present that I think this is affected by temperature. I think that in the *Selenias* of which the pupæ were kept at the lower temperature, the fore wings are longer and more angular; but on this subject I am making some exact observations, and in the meantime venture to ask the attention to it of those who have the opportunity of investigation.

The general differences in size, and possibly in shape, are, I think, exemplified by the specimens I exhibit, which were selected solely for colour and markings.

Species not found to be affected by temperature.—It may

be useful to mention the names of some species on the colouring of which I have not hitherto found any effect produced by the pupal temperature. They are the spring emergence of *P. machaon* and *P. podalirius* (both from Southern Spain), *Thais polyxena*, *A. paphia*, *D. vinula*, *T. orbona* (*comes*), and *B. cynthia*. *P. brassicæ* and *P. rapæ* are affected, but as yet I have seen no great effect produced on them. I think the *spring* emergence of *A. levana* is affected, but, so far as my opportunities have enabled me to judge, very slightly so.

Effect of light.—It has been suggested to me by members of this Society that light, especially about the time of emergence, might influence colour. I have therefore tried some experiments on the point. The first were with some of the spring emergence of *illustraria:* 32 of a healthy brood were on the 1st February divided as nearly as might be between the two sexes, and placed in lots of two pairs each on the sill inside the window of a rather large bedroom facing E.S.E., in which there was always a small fire night and morning. They were at a temperature generally ranging from about 46° to 57°, rising sometimes to 63° during sunshine. The pupæ were protected from direct sunshine. Seven lots of four pupæ each were placed in white jam-pots, their tops covered respectively with glass—clear, purple, blue, green, yellow, orange, and red; while an eighth lot was covered so as to be quite dark. Afterwards another lot of four pupæ from the same parents was similarly exposed, with no light admitted but such as came through a solution of bichromate of potash. Nearly all emerged uncrippled between 8th March and 6th April. I can find no appreciable difference between them in appearance.

Afterwards I tried the following experiments with *B. cynthia:*—On the 13th April, I took seven pupæ out of their cocoons, and laid them on the bare surface of some cocoa-nut fibre at the bottom of a very large flower-pot placed in a balcony facing E.S.E., covered with a sheet of glass, and the pupæ protected from direct sunshine. Seven others were treated in another flower-pot, side by side, with the difference that the pupæ were left in their cocoons, which were covered with tinfoil, and were also provided with long caps of tinfoil, excluding light, but capable of being pushed off by the moths in emerging, which happened. All the 14 emerged between

the 3rd and 25th July, only one being a cripple. I have failed to see any difference of appearance between the two lots. I may add that I could not find any difference in colouring produced in this species by the difference of temperature between pupæ kept at 80°, and emerging in 5 to 6 weeks, and pupæ kept in the open air and emerging in about 4 months.

General speculations as to temperature effects.—Some of the results seem attributable to the cause that a particular temperature is more conducive to health and vigour than any other, and therefore may be expected to produce larger size and greater intensity of coloration, which, in insects of the colouring of those operated on by me generally, but not always, means greater darkness. The connection between "varieties" and "cripples" is well known. A temperature of about 58° or 60° in *V. urticæ* seems to be the one most conducive to brightness and intensity of colouring and marking. And a temperature of 47°, especially if long continued, seems to stunt its size, as well as to deaden its brightness, and to produce a large proportion of cripples. In *B. quercus*, and still more in its var. *callunæ*, a temperature of 60° appears more conducive to vigour than a higher one.

But this is quite insufficient to account for all the effects produced by temperature in the cases of *V. urticæ*, *B. quercus* (and *callunæ*), *C. caja*, and *E. autumnaria*. Apart from the changes of colouring that may be supposed to be dependent on vigour, there seems in all these species what may provisionally be called a direct* tendency in the lower temperature to cause darkness, either by obscuring the general colour or increasing the size and intensity of the dark markings, or by some or all of these combined. And in the seasonally dimorphic species, such as the *Selenias* (and probably in *falcataria* also), the intensity and darkness of coloration caused by temperature appear to be quite independent of health and vigour, for those which have been forced, whether of the spring or summer emergence, appear in every way as healthy and well developed, and as thickly clothed with scales, as those kept at the lower temperature.

* In using this term I should have excluded from its application such effects in the way of darkness as can be explained by Weissmann's theory that the low temperature causes reversion to a darker ancestral form: a subject adverted to by me in earlier papers.

I have only to add that, with the permission of the authorities of the Natural History Museum at South Kensington, my exhibits will be left there for a time, for the more leisurely inspection of all interested in them, and if any desire to examine the long series I have at home, these will be much at their service.

Note.—To correct misapprehensions it may be as well to state that though, where the temperature conditions are extreme, whether high or low, there is, as might be expected, a tendency to crippling, there is no such tendency whatever under more moderate conditions. In those species in which the effects are most marked, the *extreme* effects in colouring are produced by small differences of temperature, without causing any crippling or any trace of disease or unhealthy appearance. Rather a large proportion of those subjected to extreme conditions was exhibited, as they showed much individual variation, but out of 172 specimens exhibited fully 150 are quite uncrippled.